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Short title (Running Head) : Le+Gn vs.Gn-only in BC

Keywords

Letrozole; gonadotropins; fertility preservation; breast cancer; oocyte cryostorage

Summary

Objective: To assess the advantages and disadvantages of using Letrozole for controlled ovarian stimulation (COH) in young patients with estrogen receptor-positive (ER+) breast cancer, wishing to cryopreserve oocytes.

Design: Retrospective cohort analysis.

Setting: Sixteen Italian Units for Reproductive Medicine and In Vitro Fertilization.

Methods: Data of 50 ER+ breast cancer patients undergoing COH to cryopreserve oocytes before gonadotoxic chemotherapy with a Letrozole plus gonadotropins (Le+Gn) protocol were compared with those of 25 young women with ER- breast cancer, submitted to COH using a protocol with gonadotropins alone (Gn-only).

Results: The Le+Gn protocol implied a significantly lower total Gn consumption and allowed to maintain significantly lower circulating E2 levels at all checkpoints throughout stimulation (peak E2 value 446 ± 357 vs.1553 ± 908 pg/ml, respectively; p=0.001). On the other side, the Le+Gn protocol allowed a significantly lower yield of oocytes available for cryostorage (6.6 ± 3.5 vs. 8 ± 5, respectively; p=0.038)..

Conclusions: In breast cancer patients, the association of Letrozole to Gn significantly reduces the number of oocytes available for cryostorage in comparison with the use of Gn alone. On the other side, it is associated with significantly lower E2 levels during the whole stimulation cycle, a safety issue that has been traditionally considered advantageous in case of ER+ cancers.

Introduction

Fertility preservation is an important issue for young women affected by cancer, Among malignancies that affect women in the fertile age, breast cancer is one of the most frequent; more and more frequently these patients ask to be submitted to controlled hormonal ovarian stimulation (COH) in order to retrieve and cryostore oocytes (1-3).

The available laboratory techniques to freeze and store human oocytes have reached a high effectiveness in maintaining oocyte viability and competence, However the number of eggs that are cryostored is still a critical issue in determining the chance of having a baby after cancer expecially when the slow freezing technique is applied (4) . On the other side, when the patient is affected by an estrogen-receptor positive (ER+) cancer, COH cannot be aimed exclusively at retrieving the highest number of eggs, but must even expose to the lowest possible levels of estrogens (5).

Due to its property of keeping low estradiol (E2) levels during COH, the aromatase inhibitor Letrozole has been proposed in association to Gn for oocyte harvesting in patients with E2-sensitive malignancies (6-8,11) cancer. The effectiveness of Letrozole for COH has been evaluated mainly in non-oncological patients with polycystic ovary (PCO) (12, 13), but only a few reports have studied its effectiveness in women with normal ovaries (14-16).

Our study is the first to evaluate the association Letrozole-plus-Gn (Le+Gn protocol) in comparison with the classical COH with Gn alone (Gn-only protocol) in an homogeneous cohort of young women, all affected by breast cancer. We conducted a multicenter retrospective analysis specifically focusing on two objectives: a) the protocol effectiveness in terms of oocyte retrieval and availability of eggs for cryopreservation, and b) the circulating E2 levels during COH.

Materials and Methods

Patients

Among Italian in vitro fertilization (IVF) Units that routinely use oocyte freezing for IVF patients, sixteen accepted to participate; they cryopreserved oocytes in breast cancer patients during the time period December 2000 - January 2012.

Overall, a total number of 75 young women affected by breast cancer (1-19 per IVF Unit) were included in the study; each patient was submitted to a single cycle of COH aimed at obtaining oocytes for fertility preservation. The patients' basal characteristics are shown in Table 1.

Table 1. **Patients' clinical characteristics**. Data are expressed as mean±SD.

	Le-Gn (n=50)	Gn-only (n=25)	P
Age (years)	34.4 ± 5.2	35.1 ± 4.9	ns
Patients aged ≥ 38 yrs (%)	26	36	ns
Patients with children (%)	7.5	5.2	ns
Patients with previous infertility (%)	5.8	11.5	ns
Patients with previous ovarian surgery (%)	6.8	8.6	ns
Smokers (%)	19	5.2	ns
BMI	21.8 ± 3	20 ± 1.9	ns
Basal FSH (IU/L)	7.3 ± 2.7	7.8 ± 3	ns
AMH (ng/ml)	3.9 ± 2.6	4.1 ± 3	ns
Antral Follicle Count (AFC)	10 ± 5.5	12 ± 9.9	ns

When the breast cancer was ER+ (50 patients), Letrozole was added to Gn, and the Le+Gn protocol was used; on the contrary, when the cancer was ER- (25 patients),

only gonadotropins (Gn-only protocol) were administered. The latter patients were used as a control group to assess the effects of Letrozole on COH.

Ovarian stimulation regimens

The ovarian Le+Gn stimulation (50 COH cycles) was accomplished with an antagonist protocol as described by Oktay starting always in the early follicular phase(8-9) The Gn-only stimulation regimen (25 cycles) was performed administering subcutaneous Gn (recombinant FSH or hMG) from cycle day 2 with an antagonist protocol or with a long GnRH agonists protocol. The Gn starting dose was chosen for each patient on the basis of the following clinical characteristics: age, body mass index, basal (day 3) FSH level, anti-mullerian hormone level, antral follicle count, and was then adjusted according to ovarian response. Recombinant FSH (rec-FSH; Gonal F, Merck-Serono, Geneva, Switzerland, or Puregon, MSD, Whitehouse Station, NJ, USA) was used in most stimulation cycles (82% in the Le+Gn group and 92% in the Gn-only group, respectively), whereas human menopausal gonadotropins (hMG; Ferring, Darmstadt, Germany) was used in 18% of cycles in the Le+Gn group and in 8% of cycles in the Gn-only group.

Ovarian stimulation was monitored by serial transvaginal ultrasound (US) examinations coupled to serum E2 measurements starting on stimulation day 5-8. When appropriate according to ovarian US and E2 levels, 10,000 IU of hCG (Gonasi HP, IBSA, Lugano, Switzerland) were administered and oocyte pick-up (OPU) was performed 35-37 hours later under US guidance. The retrieved oocytes were immediately observed to assess nuclear maturity, and metaphase II eggs were cryostored; the slow-freezing or the vitrification techniques (17) were used in 70% and 30% of cases, respectively.

End points and statistics

The primary end-points of the present study were: a) the number of mature, cryopreservable oocytes, and b) E2 levels during stimulation. Secondary end-points were the following: proportion of cancelled cycles, total amount of administered Gn, stimulation length, E2/retrieved and E2/cryopreserved oocyte ratios.

The Chi square test statistics was used to compare categorical covariates, and continuity correction was used to account for small expected observations. The mean levels of the studied covariates was tested by means of the Student *t* test or the Welch test according to the results of Levene's test for equality of variance. Differences were considered statistically significant at an α level <0.05.

Results

The two groups of patients did not significantly differ for any of the basal clinical characteristics (Table 1).

Overall, four cycles out of 75 were cancelled before OPU, one in the Le+Gn group and three in the Gn-only group; however, only one cycle in the Gn-only group was cancelled for inadequate ovarian response (Table 2), whereas the others were stopped for sudden health problems that forced to anticipate the oncological treatment, unrelated to the stimulation itself.

The starting Gn dose and the total dose of administered Gn were significantly lower in the Le+Gn than in the Gn-only group (Table 2).The number of developing follicles was similar with both protocols, but the number of oocytes retrieved was borderline significant in favor of Gn-only regimen and the number of mature oocytes available for cryopreservation(6.6 ± 3.5 vs. 8 ± 5; p=0.038) was significantly higher in the Gn-only group (Table 2).

The anti-estrogenic effect of Letrozole resulted in significantly lower E2 levels at all checkpoints during stimulation (Figure 1). The peak E2 level the day of hCG administration (D-hCG) was approximately one third in the Le+Gn group than in the Gn-only group (Table 2).

Discussion

The property of stimulating follicular recruitment and growth while keeping low E2 levels renders Letrozole particularly interesting for young women with E2-sensitive malignancies (e.g., endometrial and ER+ breast cancers) wishing to preserve their fertility by COH followed by oocyte or, whenever legal, embryo cryostorage (6-11, 18). On the other side the number of oocytes available for freezing is a key issue in determining the chances to preserve fertility especially with the slow –freezing technique, which was applied in the majority of cases in this studyOktay (18) reported a better oocytes recovery with the Le+Gn protocol compared with Tamoxifen-alone and Tamoxifen-plus-Gn in breast cancer patients. However a lower number of retrieved oocytes was observed in Le+Gn-stimulated breast cancer patients compared with Gn-only-stimulated healthy controls (8), and in Le+Gn-stimulated breast cancer patients compared with women with hormonal-independent cancers stimulated by Gn alone (19).

Our study is the first in which the Le+Gn stimulation regimen is compared with a Gn-only COH regimen in an homogeneous group of young women affected by breast cancer, among which the ER- subjects, stimulated by Gn-only, represent the control group.

The technical experience with oocyte freezing in Italy is one of the widest in the world (20-24) and oocyte freezing is frequently offered to oncological patients. We observed that the number of mature and oocytes, was significantly (about 40%) lower when Letrozole was used (Table 2). Although an early timing of hCG administration

as been held responsible for the higher percentage of immature oocytes in letrozole stimulation (8), in this study also the overall number of retrived oocytes was lower in patients than in controls stimulated without Letrozole.Indeed the number of oocytes obtained in our study by the Le+Gn regimen was lower than previously published (8, 19), suggesting a more cautious attitude for COH in Italy and/or a higher proportion of poorly-responding women among Italian patients. It must be remarked, in fact, that about one third of the patients included in our survey was older than 38 at the time of stimulation. the different proportion of smokers in Le-Gn and Gn-only groups and the different number of cycles receiving HMG instead of recFSH may have contributed to a lower ovarian response acting as confounders on the role of letrozole."

Above all a possible reason why in our study the oocyte yield was lower when Letrozole was used is that both the Gn starting and the Gn total doses were significantly lower in the Le+Gn group than in the Gn-only group (Table 2). Anyway, it was reported that increasing the Gn dose associated with Letrozole is not effective in enhancing oocyte yield (25); thus, the lower dose of Gn should not be responsible for the reduced oocyte yield. Alternatively, instead, it could be due to a lower ovarian responsiveness of patients with ER+ cancers in comparison to women with E2-insensitive tumors, that was reported by some (26), but not by others (27).

The number of oocytes available for freezing is indeed very important, but dealing with E2-sensitive cancers, the patient's exposure to E2 is another major issue. Estrogens are known to actively stimulate the proliferation of ER+ breast cancer cells (28,29). Until the recent past, ovarian stimulation for fertility sparing was forbidden for women with E2-sensitive cancers because of the fear of the stimulating effect of E2 on cancer progression. Indeed Letrozole-including protocols have been proposed for cancer patients just with the scope of overcoming this limitation and have been associated with a comparable cancer recurrence rate to that observed for breast

cancer patients who were not willing to cryopreserve and did not undergo ovarian stimulation (7).

Our study clearly shows that the use of the Le+Gn protocol implies a significantly lower E2 levels from the first checkpoint (day 5-8 of the stimulation cycle), throughout the whole stimulation cycle, until the day in which hCG was administered (D-hCG; Figure 1).

Moreover, we observed a significantly lower E2/oocyte ratio with the Le+Gn regimen, witnessing the lower E2 amount produced by each single developing follicle (Table 2). The observed reduction in circulating E2 in women who received Letrozole was very relevant - approximately 70% - compared to E2 levels observed in patients receiving Gn-only. Is the fear of worsening the prognosis of ER+ breast cancer patients performing a “classical” COH with Gn-only justified? Indeed it appears to be based on rather uncertain scientific data for at least two reasons: (a) it is unknown to which extent a short (some days) exposure to high E2 levels can affect the global prognosis of a woman with an ER+ breast cancer (5), and (b) it is unknown which concentration of E2 is needed to significantly accelerate ER+ breast cancer cells proliferation. On one side Letrozole administration seems reasonable in order to expose ER+ breast cancer patients to the lowest E2 levels possible, on the other side it appears questionable. Is it correct (and ethical) to apply a COH protocol that is known to have a lower chance to preserve fertility (significantly less cryopreservable oocytes) to avoid a possible risk of harmful effects of elevated circulating E2 levels? These effects have not been precisely quantified and could also be elicited by much lower E2 levels, e.g. those reached during Le+Gn COH.

With the limits of its retrospective and multicentre nature, the present study shows that in breast cancer patients the Le+Gn stimulation protocol implies a lower availability of cryopreservable oocytes than the classical Gn-only regimen, and

implies a significantly lower E2 exposure. Our observations do not allow to assess if the Le+Gn protocol is the best available option for ER+ breast cancer patients, a satisfactory compromise between safety and effectiveness. Further studies aimed at precisely assessing the impact of a short and limited E2 exposure on the kinetics of breast cancer cells and on the prognosis of premenopausal ER+ breast cancer will help to give a definite answer to this issue.

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Declaration of interest

The authors report no declarations of interest.

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